

Orphan drugs in development for urea cycle disorders: current perspectives

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Abstract: The urea cycle disorders are caused by deficiency of one of the six hepatic enzymes or two transporters involved in detoxification of ammonia. The resulting hyperammonemia causes severe brain injury unless aggressive steps are taken to reduce the accumulation of ammonia, which is thought to be the most toxic metabolite. This review describes the current state of chronic management of urea cycle disorders, focusing on new and emerging therapies. Management strategies include the mainstay of treatment, namely dietary protein restriction and supplementation with L-arginine or L-citrulline. Several currently approved medications utilize and enhance alternative pathways of waste nitrogen excretion (sodium benzoate, sodium phenylacetate, sodium phenylbutyrate in several formulations, and glycerol phenylbutyrate), working through conjugation of the drug to either glycine (in the case of benzoate) or glutamine, the products of which are excreted in the urine. Carbamoylphosphate synthetase, and thus effectively treats defective synthesis of the endogenous activator, *N*-acetylglutamate, whether due to genetic defects or biochemical inhibition of the *N*-acetylglutamate synthase enzyme. Approaches to neuroprotection during episodes of hyperammonemia are discussed, including the use of controlled hypothermia (brain cooling), as well as proposed, but as yet untested, pharmacologic therapies. Finally, cell-based therapies, including liver transplantation, infusion of fresh or cryopreserved hepatocytes, use of stem cells, and new approaches to gene therapy, are reviewed.

Keywords: urea cycle disorders, inherited hyperammonemias, orphan drugs, phenylbutyrate, *N*-carbamyl-L-glutamate

Introduction

Urea cycle disorders (UCDs) are a group of inherited defects of six enzymes and two transporters that constitute the urea cycle in the periportal liver cells. Defects in any of the enzymes or transporters lead to the respective disorders including arginase 1 deficiency, argininosuccinate lyase (ASL) deficiency, argininosuccinate synthetase deficiency, citrullinemia type 2 (caused by the defect of the citrin transporter), carbamoylphosphate synthetase 1 (CPS1) deficiency, *N*-acetylglutamate synthase (NAGS) deficiency, ornithine transcarbamylase (OTC) deficiency, and hyperornithinemia-hyperammonemia-homocitrullinuria syndrome (caused by the defect of mitochondrial ornithine transporter) (Figure 1). The main function of the urea cycle is the detoxification of ammonia and the synthesis of arginine. Defects of any of the urea cycle enzymes in the liver can result in metabolic decompensation characterized by hyperammonemia, which is life threatening if the treatment is started late or if the diagnosis is missed.¹

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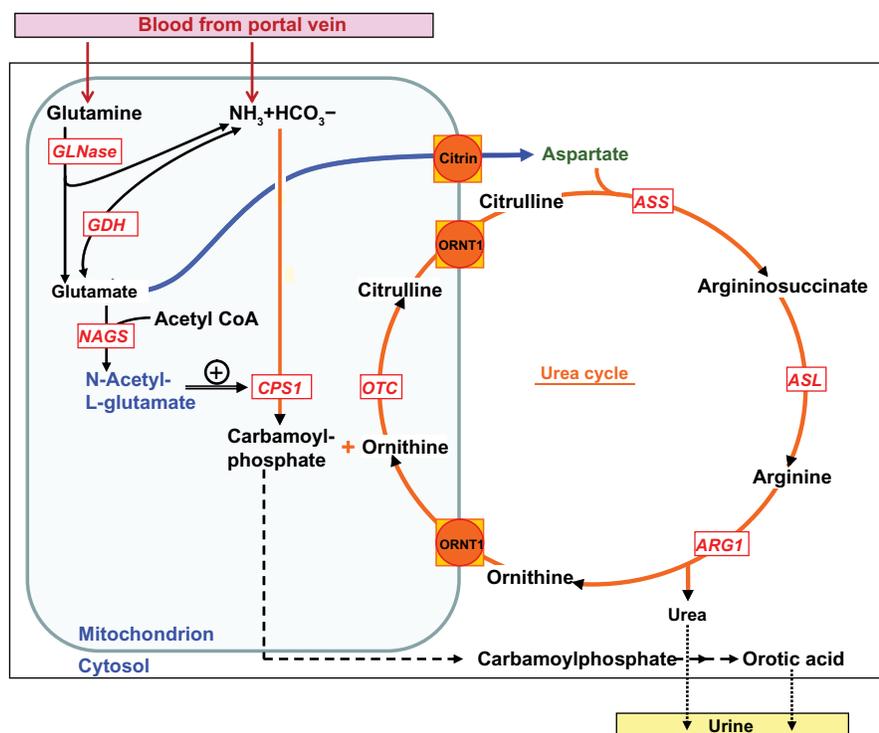


Figure 1 Enzymes and transporters of the urea cycle.

Notes: The urea cycle is shown as it is present in mitochondrion and cytosol. The encircled plus sign indicates stimulation of CPS1 by NAG. Adapted from Häberle J, Boddart N, Burlina A, et al. Suggested guidelines for the diagnosis and management of urea cycle disorders. *Orphanet J Rare Dis.* 2012;7:32. © 2012 Häberle et al.; licensee BioMed Central Ltd. Available from <http://www.ojrd.com/content/7/1/32#>.¹¹

Abbreviations: ARG1, arginase I; ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; citrin, mitochondrial aspartate–glutamate antiporter; CPS1, carbamoylphosphate synthetase I; GDH, glutamate dehydrogenase; GLNase, glutaminase; NAG, N-acetylglutamate; NAGS, N-acetylglutamate synthase; OTC, ornithine transcarbamylase; ORNT1, mitochondrial ornithine transporter.

UCDs are panethnic with variable prevalence in different populations and an overall incidence of about 1 in 35,000.² Initial presentation of a UCD can be at any age but about half of the patients present shortly after birth, triggered by postnatal catabolism.³ Other common triggering factors, characterized by endogenous protein catabolism, include viral infections and fasting. Patients with onset of symptoms outside the newborn period are classified as late-onset UCDs.^{4–6}

The most neurotoxic compound metabolites appear to be ammonia itself, which is responsible for most of the clinical symptoms and complications. The exact mechanisms of ammonia neurotoxicity are not completely understood. Different effects of accumulating ammonia on the brain include swelling of astrocytes via glutamine accumulation, energy deficit through mitochondrial dysfunction, enhanced oxidative stress due to increased nitric oxide (NO) production, increased extracellular glutamate stimulating N-methyl-D-aspartate (NMDA) receptors, inhibition of the tricarboxylic acid cycle, and secondary creatine deficiency.^{7–9}

The main goal of treatment is to avoid hyperammonemia in order to prevent neurological sequelae.

Scrupulous avoidance of acute and chronic hyperammonemia may be associated with better prognosis for patients with UCDs.¹⁰ On the other hand, the prognosis with respect to survival is commonly considered as poor in cases where the ammonia concentration in blood is >1,000 $\mu\text{mol/L}$ (reference <100 in newborns and <50 outside the neonatal period) or with duration of hyperammonemic coma >24 hours.¹¹ Infants who do survive typically have severe and permanent brain injury, thus many specialists recommend against aggressive intensive care when treatment is delayed from the time of initial presentation.

The mainstay of chronic treatment is reduction of dietary protein to the minimum required to support normal endogenous protein synthesis and normal growth, along with supplementation of essential amino acids, vitamins, and trace elements. Other common treatment strategies include the nitrogen scavenging drugs, such as sodium phenylbutyrate (NaPB), licensed in 1996 for oral/enteral use by the United States Food and Drug Administration (FDA) as Buphenyl® in the US, (Hyperion Therapeutics, Brisbane, CA, USA), and Ammonaps® in Europe (Swedish Orphan Biovitrum Ltd, Stockholm, Sweden), the new drug

formulation glycerol phenylbutyrate (GPB; FDA-approved as RAVICTI® [Hyperion Therapeutics, Brisbane, CA, USA]), sodium phenylacetic acid (NaPA), and sodium benzoate (SBZ; the combination of phenylacetic acid (PAA) and SBZ is available as an FDA-approved drug for intravenous application, AMMONUL® [Ucyclyd Pharma, Inc., Scottsdale, AZ, USA]), and the urea cycle intermediates L-citrulline (for oral/enteral use only) and L-arginine (for oral/enteral or iv use), the latter being available as free base or hydrochloride solution.^{11–13} Use of either or both of the latter two compounds, available as chemical compounds only, depends on the specific disorder and aims for excretion of citrulline (containing one waste nitrogen atom) or argininosuccinic acid (containing two waste nitrogen atoms) in the urine.

In spite of these dietary and pharmacological treatment measures, considerable risk of hyperammonemic decompensation remains, especially in the severe UCDs. Thus, liver transplantation, which is currently considered the only curative treatment, is often recommended early in the course of the disease in case of neonatal-onset UCDs or an unstable course in any patient with UCDs.^{11,14,15} Liver transplantation replaces the complete urea cycle, which is only fully expressed in liver, but other cell types that express individual component enzymes, particularly argininosuccinic acid lyase, are not corrected.

There is certainly a need for novel therapies, both for the treatment of acute hyperammonemia and for the long-term management of UCDs, since the present therapeutic situation is far from ideal. A better understanding of the pathology in ammonia (neuro) toxicity has opened the path for a variety of novel therapeutic approaches, although most have not yet added to the armamentarium of UCD drugs. First, we will describe the few recent products approved for the treatment of UCDs. Second, we will discuss novel therapeutic strategies that provide hope for an improved prognosis for individuals with UCDs in the future.

Novel drugs already brought to the market

Glycerol phenylbutyrate (RAVICTI®)

The basis to alternative pathway therapy was recognized 100 years ago with the demonstration that SBZ could divert urea nitrogen to hippurate.¹⁶ Shortly thereafter, a similar observation noted that PAA, when given orally, resulted in urinary excretion of phenylacetylglutamine.¹⁷ PB, which is converted to PAA in the liver, is now used more frequently for enteral therapy, because it has low odor and is better tolerated by patients. Since its introduction to the treatment of

UCDs,¹⁸ the use of endogenous pathways for waste nitrogen excretion has become a mainstay of management of patients with UCD.¹⁹

Recently, a new formulation of PB was brought to the market. The new drug, GPB, FDA-approved as RAVICTI®, is a pre-prodrug containing three molecules of PB joined to glycerol in ester linkage to form a triglyceride. The pre-prodrug, GPB, is hydrolyzed in the small intestine by pancreatic lipases and releases PB and glycerol. The prodrug, PB, is metabolized in the liver to the active compound PAA. Given the required GPB hydrolysis, gastrointestinal absorption of PB is slower than with NaPB.

When compared with NaPB in a Phase II study in eleven pediatric patients above age 6 years (mean, 10.2 years), GPB was shown to be at least equivalent to NaPB with respect to ammonia control.²⁰ In that crossover study, performed when the blood ammonia was clinically well controlled at baseline, switchover from NaPB to a PB-equimolar GPB dose was well tolerated and resulted in about 25% lower average ammonia exposure (assessed as ammonia 24-hour area under the curve [AUC]). Mean blood ammonia concentrations were mainly <60 µmol/L in the GPB as well as in the NaPB-treated patients, and glutamine levels did not change significantly after switching to GPB.

In a Phase III double-blinded, randomized crossover study in 44 adult patients performed consecutively, treatment with GPB was non-inferior to NaPB if ammonia control was likewise assessed as 24-hour AUC.²¹ When the results of the latter study were pooled together with data from two similarly designed short-term comparisons of GPB versus NaPB (one in pediatric patients),²⁰ ammonia 24-hour AUC and plasma glutamine were significantly lower with GPB,²¹ findings that are recently confirmed by other studies in pediatric patients with UCD.^{22,23} As a preliminary finding from one study,²¹ executive function in the study population seemed to have improved, although this observation, made after only one year of treatment, must be confirmed in future studies.

In addition to different pharmacokinetics, the main advantage of GPB from the patients' point of view may be the much improved palatability of GPB. The drug comes as an essentially tasteless liquid. Despite its oily consistency, patients have little difficulty swallowing the drug. In pediatric patients, this may result in better compliance and lessening of the burden of disease. Other potential benefits include markedly reduced volumes of drug to be consumed; one teaspoon of GPB liquid provides an equimolar dose to approximately 13 large tablets of NaPB. The sodium salt of PB also appears to cause significant gastric discomfort, which is absent in the

triglyceride (GPB), where the acidic PB is released in the small intestine and more slowly. Finally, the sodium load in therapeutic doses of NaPB is measured in gram quantities, which may contribute to multiple unpleasant effects on fluid balance and blood pressure.

Phenylbutyrate (Pheburane®)

Another formulation of NaPB was introduced to the European market in 2013 (licensed by the European Medicines Agency [EMA] as Pheburane®; Lucane Pharma, Paris, France). The drug consists of small spherical sugar cores, contributing to a sugar content of ~50% of the weight of the drug, which are coated sequentially in two separate layers with sodium PBA and ethylcellulose, the latter being a taste-masking agent for active substances.²⁴ Pheburane®, formulated as tasteless and odor-free granules, is recommended for oral use only and should not be administered by a nasogastric or gastrostomy tube because of its slow dissolution. Importantly, the sodium content of this formulation, 5.4 mmol sodium for each gram of NaPB, is identical to the other NaPB formulations available in the market.

Pharmacodynamic and pharmacokinetic properties are the same as in the active compound, PB, resulting likewise in conversion to PAA and subsequent conjugation with glutamine to form phenylacetylglutamine, which is then excreted in the urine.²⁴ Accordingly, the new formulation reduces the unpleasant taste, which should lead to improved compliance and eventually result in better efficacy of long-term treatment and better outcomes, especially in the pediatric patient population.²⁵

N-Carbamyl-L-glutamate (Carbaglu®)

N-Carbamyl-L-glutamate (carglumic acid; Carbaglu; Orphan Europe Recordati Group, Paris, France) is a licensed drug (both by the FDA and by the EMA) for the treatment of the rare NAGS deficiency.^{26–28} This defect leads to deficiency of *N*-acetylglutamate (NAG), the allosteric activator of the first urea cycle enzyme, CPS1. Carglumic acid is a chemical analogue of NAG and has been demonstrated to efficiently enter the mitochondrion. Thus, treatment with carglumic acid effectively substitutes for the NAG deficiency, although the pharmaceutical agent is not regulated by consumption of a protein containing meal in the same way as endogenous NAG. Nevertheless, NAGS deficiency is the only UCD for which an easy and highly efficacious remedy is available, which allows the patients to lead a normal life, with no need for low-protein diet or other drugs except during the periods of illness.^{11,29,30}

In addition, carglumic acid has been licensed in Europe for the treatment of acute hyperammonemia in three of the organic acidurias (methylmalonic aciduria; propionic acidemia; and isovaleric acidemia), which are inherited disorders of branched-chain amino acid degradation.^{28,31} In these conditions, hyperammonemia results from inhibition of NAGS during states of metabolic decompensation, presumably due to accumulation of specific inhibitory compounds such as methylcitrate or propionyl-CoA.

A further application of carglumic acid, the use in patients with CPS1 deficiency and potentially also in patients with OTC deficiency, has recently been suggested and is currently the subject of a multicenter Phase II study in the US. The rationale for this use is based on the assumption that additional activation of a mutant CPS1, thereby maximizing the flux through the urea cycle, may improve the overall ammonia detoxification capacity. This may be the case, in particular, if mutations affect the allosteric domain of the CPS1 protein, which comprises the C-terminal part of the protein. This theoretical concept has been supported by a number of (mainly single) patient studies in which a beneficial effect was shown.³² More recently, isotope studies performed in a small number of CPS1-deficient patients provide evidence for the efficacy of carglumic acid treatment, demonstrating increased flux through the urea cycle.³³ This is further supported by studies in insect cell-derived purified recombinant CPS1, in which carglumic acid exhibited an activity increasing effect in some of the mutants.³⁴ Notably, this effect was not only limited to mutations within the C-terminal part of CPS1 but also found in single missense changes far distant in the secondary structure of CPS1.

Likewise, for males with partial OTC deficiency, ie, with residual enzyme activity, or in female carriers of OTC mutations with hepatic mosaicism due to random X-inactivation, pharmacological upregulation of CPS1 enzyme activity may lead to increased flux through the OTC-deficient enzyme, or in cells expressing the normal enzyme in female carriers.

Drugs with a potential for development NO supplementation

The recent elucidation of a cytosolic multienzyme complex including argininosuccinate synthetase, ASL, and NO synthase,^{35,36} and evidence of NO deficiency suggested by reduced nitrosylation of marker compounds in patients with ASL deficiency, suggest an explanation for some of the hitherto poorly understood clinical findings in ASL-deficient patients, namely, arterial hypertension, chronic liver

disease, and ammonia-independent neurodevelopmental deterioration.³⁷ Importantly, impaired NO-mediated relaxation of the vasculature has been restored both in ASL-deficient mice and in a single ASL-deficient human with treatment refractory arterial hypertension by NO supplementation.³⁸ Thus, the role of NO supplementation for the subgroup of patients affected by ASL deficiency will need to be determined in clinical trials.

NMDA receptor blockade and other neuroprotective agents

Since ammonia neurotoxicity is in part mediated through activation of the NMDA receptors by glutamate, targeting this receptor by pharmacological blockade is an obvious therapeutic strategy; however, there are no research activities reported that specifically address UCDs. Thus, the reader is referred to literature on other disorders.³⁹

A number of other therapeutic targets have been inferred from research into ammonia neurotoxicity, as reviewed by Braissant et al,⁸ unfortunately, none of them have yet been evaluated in clinical trials. Nevertheless, they are listed in Table 1 to illustrate the large variety of potential therapeutic targets that may, at least in part, come into clinics in the future. Other nitrogen scavenger substances, such as L-ornithine-phenylacetate or L-ornithine-L-aspartate, have also been proposed, although possible advantages over currently existing treatments are not clear.

Table 1 Potential neuroprotective agents

Agent	Proposed mechanism of action
NOS inhibitors	To mitigate the ammonia-induced upregulation of NO ⁵⁴
Creatine	To treat cerebral creatine deficiency caused by low arginine levels as found in all enzymatic defects of the urea cycle (except ARI deficiency), to treat ammonia-induced cerebral energy deficits, and to protect axonal growth ⁵⁵⁻⁵⁷
Acetyl-L-carnitine	To treat ammonia-induced cerebral energy deficits ⁵⁸
Inhibition of CDK5/p25	To protect neurons from ammonia-induced death ⁵⁹
CNTF	To protect oligodendrocytes from ammonia neurotoxicity ⁶⁰
MAPK inhibitors	To inhibit the ammonia-induced activation of the MAPK pathway ^{60,61}
GS inhibitors	Inhibition of brain GS will reduce glutamine accumulation in astrocytes, hereby limiting osmotic cerebral edema ^{62,63}

Abbreviations: CDK, cyclin-dependent kinase; CNTF, ciliary neurotrophic factor; GS, glutamine synthetase; MAPK, mitogen-activated protein kinase; NOS, nitric oxide synthase.

Other therapeutic strategies

UCDs cannot be cured by drugs alone. It is clear that additional or alternative strategies are needed. Currently, liver transplantation by restoring the defective enzyme in the liver is the only correction for the ineffective urea cycle; thus, it can be reasonably recommended for all severe UCDs, unless there are already neurological sequelae from previous episodes of hyperammonemic decompensation rendering the extent of preservation of cognitive functions small or irrelevant.^{11,15,40} Even then, liver transplant may be considered to ease the burden of care and enhance the quality of life for affected individuals, mainly by normalizing the diet and removing the need for expensive scavenger medications and nutritional supplements, and for their families by reducing the anxiety of the constant risk of rapid metabolic decompensation. It is also possible that liver transplantation may be less expensive over the course of a lifetime than the current standard medical care. It should be remembered that liver transplant does not replace those component enzymes of the urea cycle that are expressed in non-hepatic tissues, especially argininosuccinic acid lyase, which is involved in NO synthesis. In addition, the life-long need for immunosuppression must be considered.

Because cadaveric liver transplantation is not immediately available for most patients and is technically challenging in newborns and small infants, several bridging therapies are currently being considered or already evaluated in clinical trials.

Hepatocyte transplantation

As a less-invasive but potentially effective approach, transplantation of (fresh or cryopreserved) human hepatocytes was suggested in the 1990s.⁴¹ Most researchers regard this as a potential bridging therapy for newborns with severe initial hyperammonemic decompensation at significant risk of further ammonia neurotoxicity if recurrent crises were to occur.⁴²⁻⁴⁴ This is a particularly attractive alternative for newborns in whom full organ transplantation carries high risks. Another advantage is increased availability of cells, as the cryopreserved cells can be stored until needed. It should be noted that, as in liver transplantation, there is a need for immunosuppression.

Concerns have been raised about the viability of cryopreserved hepatocytes, not only because these cells may be derived from organ donors not suitable for full organ transplantation because of long ischemia times, but also because of concerns toward mitochondrial damage due to cryopreservation.⁴⁵ The latter concern could be overcome by the use of freshly isolated cells that may have better viability.⁴⁶

At least two clinical trials are currently underway to address this approach to therapy. An ongoing multicenter, open-label clinical trial using historical controls in the US and Europe evaluating safety and efficacy of cryopreserved hepatocyte transplantation is in progress (Clinicaltrials.gov identifier NCT01195753). An additional, single-center study using freshly isolated hepatocytes transplanted into an irradiated liver to enhance engraftment and expansion of the grafted cells is also in progress (Clinicaltrials.gov identifier NCT01345578).

Stem cell transplantation

Another alternative approach, in principle with the same rationale as the aforementioned hepatocyte transplantation approach, is the proposed transfusion of stem cells.^{47–49} These cells may exhibit advantages over mature hepatocytes in terms of efficacy and may have the additional benefit of being able to be produced in large quantities in vitro.⁴⁹

Gene therapy

Gene therapy is a research focus for several inherited metabolic diseases including UCDs. In fact, in the most common UCD, OTC deficiency, a dose-escalating study was performed as early as in 1999. Unfortunately, this trial was stopped due to overwhelming, and ultimately fatal, viral sepsis from the vector in one of the subjects,⁵⁰ which put the entire field at hold for many years. Recent advances in vector biology and development have brought gene therapy back to the list of possible future therapies for UCDs,⁵¹ although the suitability of adenoviral-associated vectors for treatment of newborns remains the subject of debate. A recent review article on this topic describes the current status of, and prospects for, gene therapy for UCD treatment.⁵²

Therapeutic hypothermia

Since damage to the brain during hyperammonemia is the most relevant complication in UCDs, neuroprotective measures are warranted. Among these, therapeutic hypothermia has been proposed as potentially beneficial as it has been observed in other conditions, including traumatic brain injury or hypoxic–ischemic encephalopathy.

A recent pilot study in six newborns with UCDs, and one case with isovaleric acidemia, a classical organic acidemia, all affected by severe hyperammonemia, has proven the feasibility of adjunct therapeutic hypothermia.⁵³ Currently, a randomized multicenter study is being planned in US and European centers. This study is designed to ascertain whether treatment with hypothermia during the

initial hyperammonemic decompensation can improve the long-term neurological outcome of patients presenting with hyperammonemic crisis.

Summary

Treatment of UCDs is still very challenging and there were only few new developments in pharmacological therapy in recent years. An improvement not only of palatability but probably also of pharmacological properties is the new formulation of glycerol phenylbutyrate that acts as an alternative pathway of waste nitrogen excretion. Carglumic acid, able to activate CPS1 as the first committed step of ammonia conversion to urea, can be useful for genetic defects or biochemical inhibition of the NAGS enzyme. Other therapeutic strategies that are not yet available but subject of studies include approaches to neuroprotection (eg, the use of controlled hypothermia aiming for brain cooling), cell-based therapies (eg, infusion of fresh or cryopreserved hepatocytes, use of stem cells), and new approaches to gene therapy. Overall, novel pharmacological developments for treating patients with UCDs are much desired as the currently available treatment options are still far from being optimal. As all UCDs have low incidences, such novel therapies will require the joint interest and enthusiasm of scientists and industry dedicated to the field of rare diseases.

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Disclosure

The authors report no conflicts of interest in this work.

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